치의학박사 학위논문

Influence of saliva-coating on the ultraviolet-light-induced photocatalytic bactericidal effects on modified titanium surfaces

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이 정 은

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지도교수 안 석 준

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-ABSTRACT-

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Jeongeun Lee, DDS, MSD

Department of Orthodontics, Graduate School, Seoul National University
(Directed by Professor **Sug-Joon Ahn**, DDS, MSD, PhD*)*

The purpose of this study was to investigate the ultraviolet-light-induced photocatalytic bactericidal effects of titanium surfaces on *Streptococcus sanguinis* in the presence of saliva-coating. Three different titanium disks were prepared: machined (MA), heat-treated (HT), and anodized surfaces (AO). Each disk was incubated with whole saliva or phosphate-buffered saline for 2 hours. Antibacterial tests were performed by incubating a *S. Sanguinis* suspension with each disk for 90 or 180 minutes under ultraviolet (UV) illumination. The viable counts of bacteria were enumerated from the cell suspension and the UV-light-induced photocatalytic bactericidal effects were determined by the bacterial survival rate. Without saliva-coating, AO disks exhibited significantly decreased

bacterial survival rates compared to MA disks. The bacterial survival rates of the HT disks were intermediate between MA and AO in the absence of saliva-coating. However, saliva-coating significantly increased bacterial survival rates in all surface types. There was no significant difference in bacterial survival rates among the three surface types after saliva-coating. This study suggests that Ti-based antibacterial implant materials using TiO_2 photocatalyst may have a limitation for intraoral use.

Key words: saliva-coating, ultraviolet light, photocatalytic effect, bactericidal effect, titanium oxide

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(지도교수: 안 석 준)

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Ⅰ. Introduction

Titanium (Ti) is one of the most commonly used biomaterials in the dental field. An important feature of Ti-based biomaterials is its outstanding biocompatibility. The air-induced passivation due to the spontaneous formation of highly inert and tenacious oxide film creates a protective and stable layer of titanium dioxide (TiO_2), which minimizes ion release from the implant to the surrounding tissues. TiO_2 is found as many different crystalline forms of oxide, rutile, anatase, or brookite.[1-3] The rutile or anatase crystalline forms are most commonly seen depending on the titanium surface treatment method.[4,5]

The TiO_2 layer has been a main focus in dental research due to its photocatalytic bactericidal action.[6] Upon excitation with ultraviolet (UV) light below 385nm, the photon energy generates electron-holes pairs in the TiO_2 surface in water, which induces a series of photocatalytic reactions. The hole in the valence band can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals (OH^-). The electron in the conduction band can reduce O_2 to produce superoxide ions (O_2^-). Both the holes and OH^- radicals have a very short lifespan, but are extremely reactive with organic compounds, leading to the degradation of organic matter and bacterial death.[7-10] Recently, there have been many efforts to create antibacterial implant materials by enhancing the photocatalytic bactericidal effect of the TiO_2 layer by treatment of the titanium surface[11,12]

The titanium surfaces of implant materials are covered by saliva when exposed in the oral cavity. Given that organic film-coating, such as blood, has been cited as one of the main reasons for the clinical failure of antibacterial medical devices,[13] the clinical usefulness of the UV-light-induced photocatalytic bactericidal effects of Ti-based dental materials may be significantly influenced by saliva-coating. However, there have been few reports about the UV-light-induced photocatalytic effects of Ti-based materials in the presence of saliva-coating. The purpose of this study was to analyze the influence of saliva-coating on the UV-light-induced photocatalytic bactericidal effects of titanium disks against *Streptococcus sanguinis*, one of the primary colonizers of dental plaque on teeth. The null hypothesis of this experiment was that saliva-coating would not influence the UV-induced photocatalytic bactericidal activities of the titanium disks.

Ⅱ. REVIEW OF LITERATURE

1. Titanium and photocatalytic bactericidal effects

A. Titanium

Titanium is the ninth most abundant element in the earth's crust. Titanium (Ti), because of its good corrosion resistance, biocompatibility, and self-cleaning effect and high strength to weight ratio, is commonly used for dental implants, orthodontic brackets, wires, and temporary anchorage devices such as miniscrews.[14] There have been several reports indicating the physical properties of Ti brackets, including friction, distortion, and strength, are comparable to those of stainless steel brackets.[15-17] The corrosion resistance and biocompatibility of titanium alloys are maintained by the surface oxide layer, which mainly consists of titanium dioxide (TiO_2). Titanium dioxide (TiO_2) has been widely investigated by many research groups, because its photoactive potential is applicable to new intelligent materials, such as photocatalytic decomposition of organics and contaminants.[18] Titanium dioxide (TiO_2) also has attracted considerable attention and has been reported to be the most useful substance in organic degradation processes on account of its chemically stable properties and absence of harmful effects on human. The surface oxide film on Ti alloys generally exists in an amorphous state, which does not indicate photocatalytic activity.[4,19]

Photo-irradiated TiO_2, if the photo energies are larger than the band-gap, which causes electrons to jump to the conduction band to create holes in the valence band, generates electron-hole pairs. The generated holes are transported toward the surface because the charged carriers (electron and hole) are separated by the surface band bending and produce oxidation on the surface as a photocatalyst. Hydroxyl radicals that are extremely reactive to organic compounds are produced by the irradiation of TiO_2 at wavelengths >385 nm, resulting in photocatalytic antibacterial activity.[7,12] TiO_2 was reported to have antibacterial activity against two major bacteria in the dental field — *Lactobacillus acidophilus*[12] and *Streptococcus mutans*[20] — indicating its possible clinical applications.

TiO_2 exists in three different crystalline phases: anatase, rutile and brookite.[2] However, three crystal structures of TiO_2 - anatase, rutile, and brookite - can be produced by various oxidation methods to induce a photocatalytic reaction.[4,19] In both rutile and anatase, the position of the valence band is deep. Although both rutile and anatase have been studied for their photocatalytic activities, however, the position of the conduction band in anatase is more negative than in rutile, which results in stronger reducing power.[21] A rutile structure is more thermodynamically stable than an anatase structure, but an anatase structure is more photoactive. At more than 900 ^{o}C, an anatase crystalline structure can also be converted to rutile.[22,23] Therefore, the crystalline structure of TiO_2 is considered an important factor in the photocatalytic activity.

Oral[24] and parenteral[25] administration of TiO_2 particles indicated no toxicity symptoms or

carcinogenicity in rats. On the other hand, *in vivo* toxicity studies have demonstrated that inhalation of TiO_2 particles induces pulmonary inflammation in rats.[26,27] It was also reported that bronchioalveolar adenomas or keratinizing squamous cell carcinomas were developed when rats were chronically exposed to TiO_2.[28]

Anodic oxidation (anodizing) is a commonly used surface treatment, especially on aluminum alloys for structural application to improve the corrosion or wear resistance.[29] The application of anodic oxidation to the surfaces of titanium and its alloys is more recent. Anodisation of titanium at room temperature forms titanium dioxide on the surface, which is predominantly anatase. Since the microbial fouling of titanium surfaces is the major problem with respect to the use of titanium in the sea water cooled condensers of power plants, the self-sterilizing ability of anatase type of TiO_2 thin films yielded the idea of growing a thin film of the anatase on the biofouling prone titanium surface to reduce the attachment of these organic living cells, using the above mentioned industrially feasible process of anodisation.

Yu et al.[30] have reported that specific photocatalytic activity decreases with increasing thickness of titanium dioxide layers and that photocatalytic activity depended on many factors such as the distance to which the reactant should reach to capture the electrons/holes generated in TiO_2 thin films, the amount of hydroxyl ions per unit weight TiO_2, film thickness, average grain size and so on.

B. Photocatalytic bactericidal effect of titanium dioxide

The use of TiO_2 as a photocatalyst for the decomposition of organic compounds and microbial organism including viruses, bacteria, and cancer cells has been reported,[31] as well as its potential use in sterilization of medical devices, food preparation surfaces, air-conditioning filters, and sanitary-ware surfaces.[14,32] Shieh et al. have reported an antibacterial performance of TiO_2 against *Escherichia coli* that could reach 99.99% bacterial reduction under an activation by visible light.[33] Its bactericidal effect was also observed on food-pathogenic bacteria such as *Salmonella choleraesuis*, *Vibrio parahaemolyticus*, and *Listeria monocytogenes*,[34] as well as *pseudomonas aeruginosa*.[35]

Matsunaga *et al.* reported for the first time in 1985 the antibacterial effect of TiO_2 photocatalytic reaction.[36] Today, photocatalytic antibacterial tiles, antifogging glass and air cleaners are among the commercial applications of the photocatalytic activity of TiO_2 based on its self-cleaning ability.[37] Direct irradiation with ultraviolet C (UVC) rays (254 nm) is a possible method of disinfection. However, since this type of radiation is injurious to health, it involves occupational medicine risks. In addition, this type of radiation is effective only when applied directly; fields obscured by other obstacles in the area irradiated – e.g. in pits in the materials used – remain untreated.

A potential alternative may be provided by substrates made of light-guiding material, coated with

specific semiconductors and stimulated by indirect mild ultraviolet A (UVA) light (320-400nm). This method shows oxidative and disinfectant activity. The semiconducting material about which most information is available is titanium dioxide (TiO_2). A recent review article provides a comprehensive report of the mechanism involved and the potential fields of application.[7] There is now wide agreement regarding the mechanism: in TiO_2, an electron is transferred from the valence band to the conduction band by absorption of a photon, and the resulting electron hole pair reacts with molecules on the surface of the semiconductor. Various reactive oxygen radicals caused by reactions of the hole have been identified in aqueous solution, mainly the OH radical.[38,39] The free electron simultaneously created reacts with dissolved oxygen, to produce among other things hydrogen peroxide. These reactive species can oxidize organic material up to complete mineralization, depending on the experimental conditions.[7] Overall, the organic molecules react with dissolved oxygen to produce CO_2 and H_2O. During photocatalytic oxidation of *Escherichia coli*, Jacoby et al.[40] measured the CO_2 released and found 54% mineralization within 75 hours.

Photocatalytic activity strongly depends on the surface redox potential, the band-gap and the lifetime of photo-generated electron-hole pairs. Anatase, which has a larger band gap, tends to increase the surface redox potentials and prolong the carrier lifetime in comparison with rutile.[41]

The fact that the OH radicals released from the photocatalytic TiO_2 can decompose organic

compounds and damage the cell walls of microorganisms may imply a negative supposition. Hydroxyl radials from TiO_2 may also affect normal oral epithelial cells. However, this serious defect could be excluded by a simple solution. The low efficiency for the utilization of visible light and the relatively low intensity of UV light in normal daylight are the major limiting factors for the use of TiO_2 but are an advantage in this case. As described above, the photocatalytic activity of TiO_2 is usually effectively activated by UV light with a wavelength less than 380nm. Therefore, the photocatalytic activity can be modulated by manually controlling the illumination time and period in dental clinics.[42] There have been various reports on the antibacterial effect of TiO_2. For example, Kühn et al[11] evaluated the antibacterial effects of a TiO_2 coating on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Candida albicans*. They reported that the antibacterial effect is in the order of *E. coli* > *P. aeruginosa* > *S. aureus* > *E. faecium* > *C. albicans* and concluded that the order was mainly determined by the complexity and density of the cell wall. It is reported that efficiency of photocatalysis was in the order of virus > bacterial cell > bacterial spore and that the susceptibility varies by microorganism type, in particular, cell wall thickness.[31] Another report suggested that the difference in the resistance to oxidative damage depends on the type of microorganism.[43] Their conclusion is based on the fact that UVA on *Enterobactercloacae* was less effective in growth inhibition than for *E. coli* or *P. aeruginosa*.

The TiO_2 photocatalyst has not only potent decomposing power, but also a superhydrophilic property,

and consequently a self-cleaning effect. Because of its wide range of functions, the TiO_2 photocatalyst is rapidly finding applications in various fields. It is used for antibacterial building materials, indoor and outdoor lamps, contact lenses and medical appliances. If TiO_2 films are prepared with a certain fraction of SiO_2, it acquires superhydrophilic properties. The surface is hydrophobic before UV illumination, but illuminated, oxygen atoms are ejected creating oxygen vacancies in the reduction process that are occupied by water molecules. After TiO_2 was exposed to UV light, the contact angle of water droplets decreases to essentially zero degrees to enhance wettability.[44] In other words, pollutants on the surface can be washed away with water.

2. Saliva

Saliva contains water, proteins, electrolytes, gingival sulcular fluid, serum, cells, bacteria, food residue, and organic molecules. It also includes a range of antioxidants. Antioxidant agents can be classified into three groups according to their function. First, preventive anti-oxidants inhibit the formation of free radicals, including superoxide dismutase (SOD), carotenoids, catalase, glutathione peroxidase, transferrin, albumin, and haptoglobin. Secondly, radical-scavenging anti-oxidants, such as vitamin A, vitamin E, uric acid, ascorbic acid, albumin, and bilirubin, eliminate free radicals to inhibit the initiation and propagation of free radical damage. Subsequently, DNA repair enzymes, protease, transferase, lipase, and repair and de novo enzymes heal injuries and reorganize the structures of the membrane.[45] Those antioxidants in

saliva could function as blockers in the photocatalytic bactericidal effect of titanium dioxide by removing the reactive oxygen species (ROS) from the titanium oxide surfaces.

The terms “whole saliva”, “mixed saliva” and “oral fluid” are used to describe the combined fluids present in the oral cavity. This fluid is mainly composed of water (99.5%), proteins (0.3%) and inorganic and trace substances (0.2%).[46] The proteins in saliva (1–2 mg/ml) are mainly constituted by glycoproteins,[46] enzymes (e.g., a-amylase, carbonic anhydrase), immunoglobulins, and a wide range of peptides (cystatins, statherin, histatins, proline-rich proteins) with antimicrobial activities.[46,47] The inorganic fraction of saliva contains the usual electrolytes (sodium, potassium, chloride and bicarbonate) of the body fluids but at different concentrations making saliva a hypotonic fluid.

There is no doubt that pellicles, derived from saliva, play a significant role in the maintenance and microbial colonization of oral surfaces.[48] Salivary pellicles are not limited to tooth surfaces. They also form on the oral mucosal epithelium,[49] dental appliances and restorations,[50] titanium implants,[51] and orthodontic appliances.[52] In particular, the interactions between salivary components in the pellicles and micro-organisms affect initial microbial adherence, which is recognized as a primary step of plaque formation and associated disease.[53]

The design of oral implants or miniscrews requires communication with the oral cavity through the

mucosal or gingival tissues. Exposure in the mouth presents a unique surface that can interact with host indigenous bacteria, leading to plaque formation. Gibbons and Van Houte[54] noted that the interaction of host flora with the teeth involves a highly selective process related to specific interactions between tooth-bound salivary pellicles and bacterial surface adhesions. Alterations in either salivary pellicles or the bacterial surface can modify the initial bacterial attachment to that surface and ultimately change the potential to develop plaque-derived periodontal disease. The ion distribution and charge character of titanium should be different from enamel. Therefore, salivary pellicle formation at each of these surfaces may, in turn, be different.

Ⅲ. MATERIALS AND METHODS

1. Material preparation

The disks (10.0 mm in diameter and 3.0 mm thick) were fabricated from grade 4 commercially pure titanium. Three different surfaces were prepared: machined surface (MA, no surface treatment), heat-treated surface (HT), and anodized surface (AO). The HT disks were heat-treated at 300^{o}C for 100 minutes in air. The AO disks were anodized in an electrolyte solution containing 0.35 M sulfuric acid and 0.35 M phosphoric acid at 50V. All disks were packed, sealed and sterilized with ethylene oxide gas. For each surface treatment group, 40 disks were fabricated.

2. Surface analysis

The surface roughness of each sample was measured using a confocal laser scanning microscope (Axiovert 200M, Carl Zeiss, Thornwood, NY, USA). The multi-argon laser emits light at a wavelength of 633 nm and allows for the calculation of the arithmetic mean surface roughness from a mean plane within the sampling area (900 × 900 × 80 μm). The system provides the numerical values for the surface roughness parameter, which is defined as the arithmetical mean deviation of the assessed profile. Each

surface roughness reading was performed three times on three different areas for each disk. The phase components were analyzed using thin-film x-ray diffractometry (TF-XRD), which was used to identify the crystal structure of TiO_2 in a scanning range of $2\theta = 20° - 70°$.

3. Saliva preparation

Unstimulated whole saliva was collected from 6 healthy volunteers. The research protocol was reviewed and approved by the Institutional Review Board of Seoul National University Dental Hospital (CRI11025). The volunteers had no acute dental caries or periodontal lesions. Saliva collection was routinely performed between 9:00 A.M. and 11:00 A.M. to minimize the effects of diurnal variability on salivary composition. The saliva samples were centrifuged at 3,500 x *g* for 10 minutes to remove any cellular debris and the resulting supernatant was used after filter-sterilization through a Stericup & Steritop (Millipore, Billerica, MA, USA). The saliva was stored at -20^{o}C before use.

4. Bacterial culture

Overnight cultures of the *Streptococcus sanguinis* SL1 strain were transferred to pre-warmed brain heart infusion (BHI) (Difco, Sparks, MD, USA) medium and grown at 37^{o}C in a 5% CO_2 chamber to OD_{600} of 0.5. Cells were washed twice with phosphate-buffered saline (PBS, pH = 7.2) and resuspended

to an OD_{600} of 0.5 (approximately 3.5 X 10^7 colony forming units/mL).

5. Saliva treatment

Each titanium disk was placed in polystyrene 48-well cell culture clusters (Corning Inc., Corning, NY, USA). The disks were divided into two groups: saliva-coating or no saliva treatment. For the saliva-coating group, each disk was conditioned with 500 μL saliva in the well at 37°C for 2 hours with gentle shaking, followed by two washes with PBS. After air drying for 30 minutes, 500 μL of the cell suspensions (OD_{600} = 0.5) were inoculated into the wells. For the group that received no saliva treatment, the same procedure was performed with sterile PBS instead of saliva.

6. Evaluation of bactericidal effects under UV Illumination

The cell culture clusters were monitored with or without UV illumination. For the UV illumination group, the cell culture clusters were incubated for 90 or 180 minutes under UV light using a type F15T8BLB black light blue lamp (SANKYO DENKI**,** Kanagawa, Japan). The light intensity was 2.0 mW/cm^2 at a peak wavelength of 352 nm. The light source was placed 10 cm above the samples. For the group without UV illumination, UV light was blocked with aluminum foil during the experiment.

After incubation, each bacterial suspension was transferred into a 1.5 mL Eppendorf tube. The collected cell suspensions were serially diluted, plated on BHI agar, and incubated at 37°C for 2 days before viable cells were counted. Cell counts recorded in colony forming units (CFUs). All assays were performed in duplicate and repeated seven times.

The UV-light-induced photocatalytic bactericidal effects were determined by the bacterial survival rate using the following equation: bacterial survival rate (%) = (viable cell count with UV irradiation/viable cell count without UV irradiation) × 100.

7. Preparation of the blank group

The same experiments were performed using the same cell clusters without titanium disks (blank group). Each well of the cell culture clusters was conditioned with 500 μL of saliva or PBS for 2 hours in the absence of titanium disks. The bacterial survival rates in the blank group were compared to those in the experimental groups containing titanium disks.

8. Statistical analysis

Surface roughness was analyzed using one-way ANOVA to compare the differences among the three surface types. Factorial ANOVA was used to analyze the differences in viable cell counts and bacterial

survival rates on cell suspension with respect to surface types, saliva-coating, and incubation time. Bonferroni's t test was used as post-hoc test. All values were considered significant when $P < 0.05$.

Ⅳ. RESULTS

There were significant differences in surface roughness among the three surface types. HT and AO disks had rougher surfaces than MA disks (Table 1). The TF-XRD spectra of the titanium disks are shown in Fig. 1. The AO group exhibited TiO_2 peaks corresponding to a 2 θ value of 25.2 indicating the presence of an anatase structure, while the HT group showed TiO_2 peaks corresponding to the presence of a rutile structure with a 2 θ value of 27.446 in the TF-XRD spectra.

Table 2 shows UV-induced changes in the viable cell counts of *S. sanguinis* with respect to surface type, illumination time, and saliva-coating. Generally, extended UV illumination time significantly decreased viable cell counts. The viable cell counts after 180 minutes illumination was significantly decreased compared to those after 90 minutes illumination. There were profound differences in viable cell counts among the surface types. UV illumination greatly decreased the viable cell counts in the AO group compared to those in the MA and blank groups. The viable cell counts in the blank group were higher than those in the experimental groups containing titanium disks in the absence of saliva-coating.

Cell counts in the HT group were intermediate between AO and MA, but there was no significant difference in the absence of saliva-coating. Although saliva-coating significantly increased viable cell counts of all the experimental groups, the differences in viable cell counts among the three surface types were decreased after saliva-coating (Table 2). After saliva-coating, the viable cell counts in AO groups

demonstrated statistical differences with those in the blank groups after 180 minutes UV illumination. Without UV illumination, however, the viable cell counts were not significantly influenced by surface type, illumination time, or saliva-coating (Table 3). The viable cell counts in blank groups also showed similar results with other surface type.

To demonstrate the UV-induced photocatalytic bactericidal effects of the different titanium surfaces, the UV-induced bacterial survival rates of the experimental groups (containing titanium disks) were compared to those of the blank group (Blank) (Fig. 2). In the absence of saliva-coating, the bacterial survival rates were lower in the AO and HT groups than in the blank group, while there were no significant differences in the bacterial survival rates between the MA and blank groups. There was also significant difference in bacterial survival rates between the AO and MA groups in the absence of saliva-coating (Figure 2). However, saliva-coating significantly increased the bacterial survival rates in the blank group as well as in all of the experimental groups. In addition, saliva-coating significantly decreased the differences in bacterial survival rates among the groups. As a result, the differences in bacterial survival rates were eliminated between the MA and AO groups and between the HT and blank groups in the saliva-coating groups (Fig. 2).

Ⅴ. DISCUSSION

UV light is a collective term for electromagnetic waves with a wavelength range of 110 - 380 nm. It is broadly classified into UVA, UVB, and UVC according to wavelength.[4] UVA which has a peak emission of approximately 352 nm, was used in this study, because UVA illumination has some deleterious effects on bacteria,[6,55] but causes relatively little damage to the organisms compared to UVB and UVC illumination.[20] The germicidal effects of UVA illumination have been demonstrated and proven for a wide range of microorganisms including oral bacteria.[56,57] Its biological effects are largely the result of energy transferred through generating active oxygen intermediates, such as superoxide ions, hydroxyl radicals, and hydrogen peroxide from water, which causes damages to bacteria.[56] This study also showed that UVA illumination itself induces bactericidal effects on *S. sanguinis* in the blank group, although bacterial survival rates in the blank group were higher than those in the experimental titanium disk groups (Fig. 2).

The primary effect of surface treatment on titanium materials, such as anodizing and heat treatment, is to increase the surface area, and thereby change the surface from low energy, hydrophobic state to high energy, hydrophilic state. Surface treatment stimulates cell attachment, differentiation, and the formation of the extra matrix, which can accelerate the osseointegration of implants.[58] Recently, such surface treatment has been reported to have a significant influence on the UV-light-induced photocatalytic

bactericidal effect through changes in the surface area, crystallinity, and crystal structure of the titanium materials[4, 59].

This study also showed the different UV-induced photocatalytic bactericidal effects of TiO_2 according to surface type. In the absence of saliva-coating, the AO and HT groups exhibited higher UV-induced bactericidal effects than the blank group, while the MA group did not demonstrate any specific UV-induced bactericidal effects compared to the blank group (Fig. 2). In particular, the AO group tended to exhibit stronger photocatalytic bactericidal effects than HT, even though there were no significant statistical differences between the AO and HT groups in bacterial survival rates. However, there were significant differences in the viable cell counts between the AO and HT groups after 180 minutes UV illumination without saliva-coating (Table 2). These results demonstrated the strongest photocatalytic bactericidal effect of AO groups compared with other surface modification groups. The stronger effects in the AO groups are due to anatase structures in AO, which have a larger band gap, increased surface redox potentials, and prolonged carrier lifetime in comparison with the rutile and brookite structures.[60] This is consistent with findings from previous studies, which showed that both anatase and rutile structures have UV-induced photocatalytic activities, but anatase is a more efficient photocatalyst compared to other forms of titanium oxide such as rutile and brookite[4, 61, 62].

The photocatalytic effect is enhanced by an increase in surface area because increased surface area

provides more available space for the production of active oxygen.[63] The higher photocatalytic bactericidal effects of the AO and HT groups versus the MA group may partly be due to the rougher surfaces of the AO and HT groups compared to the MA group (Table 1 and Fig. 2).

The hypothesis of the present study was rejected because the UV-induced photocatalytic bactericidal effects of TiO_2 were significantly influenced by saliva-coating (Fig. 2**).** Saliva-coating greatly decreased the viable cell counts in all conditions and increased the bacterial survival rates of the MA, HT, and AO groups. In addition, the differences in the bacterial survival rates between the MA and AO groups and between the HT and blank groups were eliminated after saliva-coating. Under no saliva treatment condition, AO groups showed the photocatalytic bactericidal effects after 90 and 180 minutes UV illumination. However, after saliva-coating, the viable cell counts were increased in all surface types. As a result, there was no significant difference in the UV-induced photocatalytic bactericidal effects among the three titanium surface modification types after saliva-coating. These results indicate that saliva-coating significantly inhibits the UV-induced photocatalytic bactericidal effects of TiO_2.

There are multiple explanations for the inhibition of bactericidal effects by saliva-coating. First, saliva-coating may inhibit the germicidal effects by UV illumination itself, which is confirmed by the fact that saliva-coating significantly increased the UV-induced bacterial survival rates in the blank group (Fig. 2). Saliva contains a range of antioxidants, such as superoxide dismutase, carotenoids, catalase, glutathione

peroxidase, transferrin, albumin, and haptoglobin, which are known to inhibit the formation of free oxygen radicals.[45] The antioxidants in the saliva-coating may decrease the UV-induced bactericidal effects by preventing the initiation and propagation of UV-induced free radical production in cell suspensions. Second, saliva-coating acts as a barrier on titanium surfaces, which can inhibit UV-light-induced production and/or diffusion of active free radicals by TiO_2 photocatalysts. Because of the short half-life and low diffusion potential of hydroxyl radicals and superoxide ions, bacterial targets to be oxidized must be close to the area where active free radicals are generated, that is, near the titanium disk surfaces.[20] Therefore, the interruption of production and/or diffusion of active radicals by saliva-coating may result in decreased photocatalytic bactericidal effects of TiO_2 around the titanium surface.

Given that saliva is continuously produced in and secreted from salivary glands, and saliva-coating significantly decreased the UV-induced photocatalytic bactericidal effects of TiO_2, Ti-based antibacterial appliances using TiO_2 photocatalysts may have a limitation for intraoral use. Further in vivo studies are necessary to determine the effectiveness of the UV-induced photocatalytic antibacterial dental materials in the oral cavity.

Ⅵ. CONCLUSIONS

In this study, the UV-induced photocatalytic bactericidal effects of TiO_2 were investigated in the presence of saliva-coating. In the absence of saliva-coating, AO and HT disks exhibited stronger UV-induced photocatalytic bactericidal effects than MA disks. However, saliva-coating significantly inhibited the photocatalytic bactericidal effects of TiO_2 and eliminated the differences in the photocatalytic bactericidal effects of TiO_2 among the different surface modifications. The results of this study suggest that the UV-induced photocatalytic effects were significantly influenced by the presence of saliva-coating as well as by the crystal phase of the titanium.

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TABLES

Table 1. Surface roughness of each titanium disk used in this study

	Surface type			Multiple comparisons[d]
	MA[a] (Mean±SD)	HT[b] (Mean±SD)	AO[c] (Mean±SD)	
Surface roughness (μm)	0.57 ± 0.14	1.21 ± 0.15	1.05 ± 0.13	MA < (AO , HT)**

[a]MA, Machined titanium surface

[b]HT, Heat-treated titanium surface

[c]AO, Anodized titanium surface

[d]One-way ANOVA with *t* tests using the Bonferroni method was used to analyze the difference in surface roughness among three groups at a significance level of α=0.05.

[*]Significance value ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: not significant

Table 2. Viable cell counts of *Streptococcus sanguinis* suspension containing variously treated titanium disk after UV illumination (90 or 180 minutes). Cell counts were expressed as colony forming units (CFUs).

Saliva treatment	Time	Surface type (x10^7 CFUs)				p-value	Multiple comparisons[4]
		Blank (Mean±SD)	MA[1] (Mean±SD)	HT[2] (Mean±SD)	AO[3] (Mean±SD)		
No saliva treatment	90 min	2.09±0.33^{a}	1.94±0.33^{a}	1.71±0.35^{a,b}	1.51±0.51^{b}	0.0011**	90 min > 180 min***
	180 min	1.07±0.27^{a}	0.87±0.16^{b}	0.78±0.13^{b}	0.54±0.12^{c}	0.0000***	
Saliva coating	90 min	2.30±0.15^{a}	2.39±0.53^{a}	2.25±0.49^{a}	1.75±0.41^{b}	0.0012**	No saliva treatment < saliva-coating***
	180 min	1.64±0.51^{a}	1.39±0.40^{a,b}	1.35±0.35^{a,b}	1.19±0.25^{b}	0.0351*	

[1]MA, Machined titanium surface

[2]HT, Heat-treated titanium surface

[3]AO, Anodized titanium surface

[4]Bonferroni's t test was used as post-hoc test at a significant level of $\alpha = 0.05$.

The same superscripts indicate no statistically significant difference between the indicated groups ($p > 0.05$).

[*]Significance value ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: not significant

Table 3. Viable cell counts of *Streptococcus sanguinis* suspension containing variously treated titanium disk without UV illumination. Cell counts were expressed as colony forming units (CFUs).

Saliva treatment	Time	Surface type (x10^7 CFUs)				p-value	Multiple comparisons[4]
		Blank (Mean±SD)	MA[1] (Mean±SD)	HT[2] (Mean±SD)	AO[3] (Mean±SD)		
No saliva treatment	90 min	3.26±1.73	3.01±1.08	2.81±0.89	2.73±0.94	0.5181^{NS}	Blank = MA = HT = AO
	180 min	2.91±0.84	2.83±1.00	3.00±1.11	2.69±0.83	0.6600^{NS}	No saliva treatment = Saliva coating
Saliva coating	90 min	2.72±1.20	2.86±1.06	3.01±1.04	2.78±1.08	0.7306^{NS}	
	180 min	3.40±1.37	3.05±1.10	2.89±0.97	2.77±0.87	0.4388^{NS}	90 min = 180 min

[1]MA, Machined titanium surface

[2]HT, Heat-treated titanium surface

[3]AO, Anodized titanium surface

[4]Bonferroni's t test was used as post-hoc test at a significant level of $\alpha = 0.05$.

[*]Significance value ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: not significant

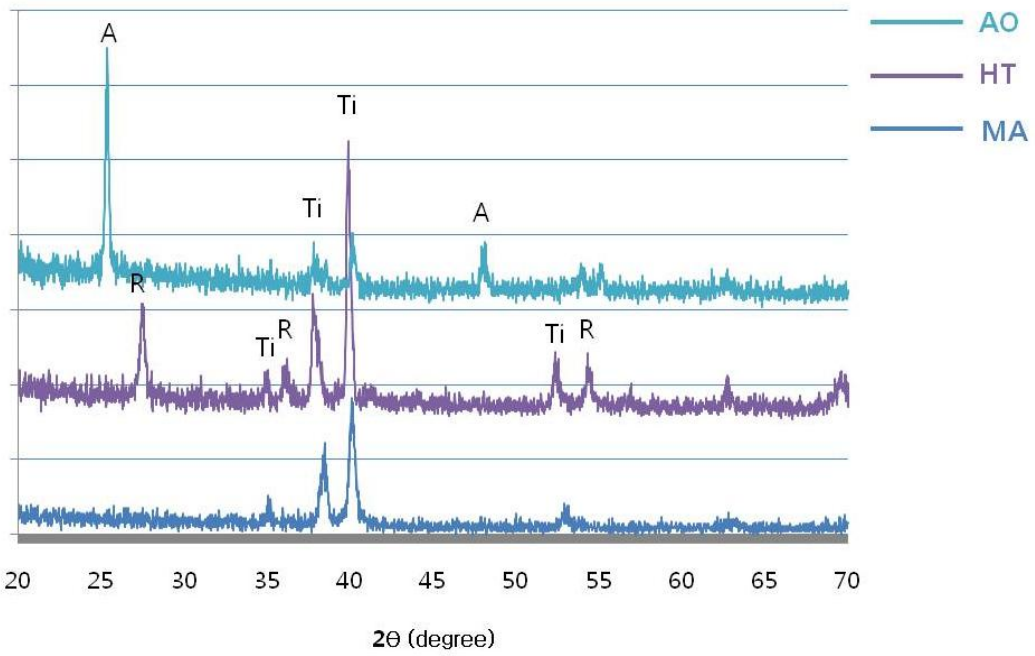

Figure 1. The X-ray diffractometry spectra of the titanium disks with the machined titanium surface (MA), heat-treated titanium surface (HT) and anodized titanium surface under 50 V (AO). Rutile peaks (R) appeared on the HT disks and anatase peaks (A) were observed on the AO disks. MA disks exhibited only Ti peaks (Ti).

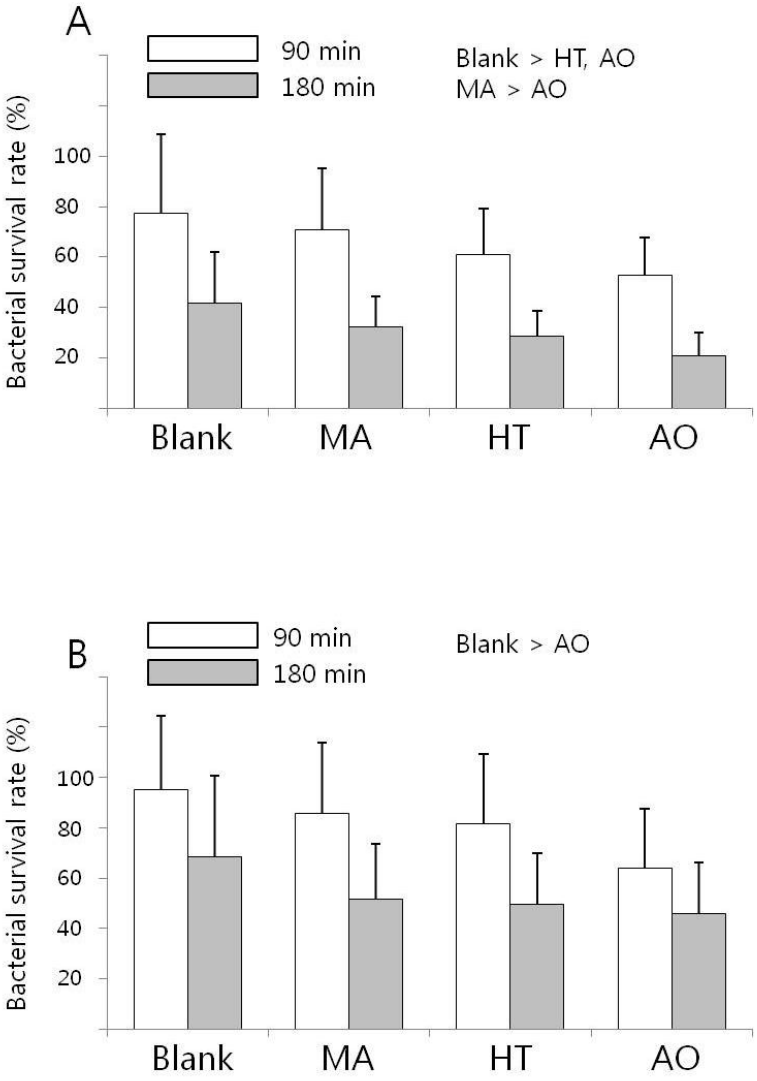

Figure 2. The UV-induced bacterial survival rates of the experimental titanium disk groups (machined titanium surface [MA], heat-treated titanium surface [HT], and anodized titanium surface under 50 V [AO]) and the blank group without titanium disks (Blank) in the absence of saliva-coating (A) or in the presence of saliva-coating (B). The bacterial survival rate was defined by the following equation: bacterial survival rate (%) = (viable cell count with UV irradiation/viable cell count without UV irradiation) × 100.

국문초록

표면처리된 티타늄에서 타액 코팅이 자외선에 의한 광촉매 살균효과에 미치는 영향

서울대학교 대학원 치의과학과 치과교정학 전공

(지도교수: 안 석 준)

이 정 은

본 연구의 목적은 타액 코팅이 자외선 조사에 의한 티타늄 표면의 *Streptococcus sanguinis*에 대한 광촉매 살균효과에 미치는 영향을 평가하는 것이다.

티타늄 디스크를 표면 처리하여 절삭처리군(Machined titanium surface, MA), 열처리군(Heat-treated titanium surface, HT), 양극 산화처리군(Anodized titanium surface, AO)으로 분류하였다. 각각의 디스크를 비자극성 전타액(unstimulated whole saliva)과 PBS(phosphate-buffered saline)에 2시간 동안 배양한 후, 광촉매 살균효과를 평가하기 위해 티타늄 디스크를 *Streptococcus sanguinis* 배양액에 넣고 자외선을 90분 혹은 180분 동안 조사하였다. 살아있는 세균 수는 세포 배양액에서 단계희석법을 통하여 계산하였고, 세균의 생존율을 이용하여 자외선에 의한 광촉매 살균효과를 평가하였다.

타액 코팅이 없을 때는 AO 군이 MA 군에 비해 세균의 생존율이 유의하게 낮았으며, HT 군의 세균 생존율은 MA 군과 AO 군의 중간이었다. 그러나 타액 코팅 군에서는 모든 표면처리 군에서 세균 생존율이 유의하게 증가하여 표면처리 군 간의 세균 생존율에서 유의한 차이가 없었다. 본 연구는 타액이 존재하는 구강 내 상황에서 자외선을 이용한 티타늄 표면에서의 광촉매 살균효과를 기대하기 어렵다는 것을 보여준다.

주요어: 타액 코팅, 자외선, 광촉매 효과, 살균 효과, 이산화 티타늄

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